1216

BROWNING OF SUGAR SOLUTIONS

I. Effect of pH and Type of Amino Acid in Dilute Sugar Solutions[a]

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Many years of research on the browning of food materials has shown that, under favorable conditions for each, many organic compounds react in various ways to form dark-colored products. After Maillard *(5)* published his classical work on the reaction of amino and carbonyl groups, most of the work on browning was directed toward finding the mechanism of the formation of the colored reaction products of amino compounds and reducing sugars, stressing the role of the amino compounds. That little study has been directed toward other reactants of the browning mechanism is indicated by excellent reviews of the subject which have been published by Hodge *(2)* and Liggett and Deitz *(4)*. More recently, however, Schroeder, Iacobellis and Smith *(6)* have proposed the theory that browning and the Maillard reaction may occur independently of each other. They found that browning (expressed in qualitative terms) in glucose solutions appeared to be largely due to the effect of pH on the hexose, and not to the interaction between amino compounds and carbohydrates. Taufel and Iwainsky *(7)* have reported similar results. Joslyn *(3)* has shown that the amino acids play a minor role in browning.

Since the typical brown color of maple sirup is produced in the presence of almost no amino compounds (0.001% *N* in maple sap), a type of nonamino browning is indicated. Therefore, studies at this Laboratory on the control of color and on the development of flavor in maple sirup have been directed primarily toward the sugars, which are the major constituents of maple sap and sirup. It is quite possible that alkaline degradation of the hexoses in the sap is part of the mechanism of color development in maple sirup, since the sap passes through a stage of alkaline pH, during the evaporation *(1)*. As to the amino-carbonyl reaction, it is well known that this occurs best in concentrated solutions or in the dry state, whereas the browning of maple sirup takes place in a dilute solution.

In an effort to clarify the browning mechanism as it applies to maple sap, the authors have for some time been conducting experiments with model systems of dilute glucose solutions both with and without amino acids and under a variety of pH conditions. This paper presents the results of some of these studies on the effect of pH and type of amino acid on the browning of dilute glucose solutions. Statistical analysis of the data provide further evidence to substantiate the theory that the effect of pH on the hexoses in the sap are primarily responsible for the browning reaction in maple sirup.

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EXPERIMENTAL

Effect of pH and individual amino acids in glucose solutions. To a series of 48 portions of 10 ml. of distilled water in 50-ml. beakers was added 1 ml. of a 1.25 *M*. glucose solution. The series was divided into 4 sets of 6 solutions each in duplicate. To each of the portions in 3 sets, 5 ml. of 1.25 *M*. solutions of L-lysine monohydrochloride, DL-alanine, and L-glutamic acid monohydrochloride were added respectively. The fourth set, the control, contained only the glucose. The solutions in each of the 4 sets were adjusted to the desired pH (3, 5, 6, 7, 8, and 9 ± 0.05) by titration with either 1 *N*. sodium hydroxide or 1 *N*. phosphoric acid. Immediately 15 ml. of the appropriate phosphate buffer was added to maintain the desired pH constant throughout the heating period. The buffered solutions were quantitatively transferred to 50-ml. volumetric flasks and made to volume with distilled water. The solutions were 0.025 *M*. in respect to glucose and 0.125 *M*. in respect to the amino acid. Ten milliliters of each of these solutions were transferred to a 16 mm. x 15 cm. test tube (drawn down to 2 mm. 5 cm. from the open end of the tube). To fill the tubes they were first warmed and then the 10 ml. of solution was added to the top funnel-like portion. The tube was then chilled in ice water and the solution drawn into the lower part of the tube with 2 cm. of free space between the liquid and the constricted portion. The tubes were allowed to drain a few minutes, after which the constricted portion was sealed off with a flame.

The glucose solutions in the sealed tubes were heated at 114° C. by autoclaving at 10 p.s.i. for 20 minutes. Following this, the tubes were immediately chilled in ice water. The tubes were opened at the seal and aliquots removed for measurement of color.

TABLE 1

Effect of individual amino acids and pH on the color developed in a 0.025 *M*. glucose solution heated for 20 minutes at 114°C. in an autoclave

Components	pH	Absorbance 500 mμ
Glucose	3	0.00
	5	0.00
	6	0.00
	7	0.00
	8	0.18
	9	0.48
Glucose + DL-alanine	3	0.00
	5	0.00
	6	0.00
	7	0.14
	8	0.20
	9	0.33
Glucose + glutamic acid	3	0.00
	5	0.00
	6	0.00
	7	0.02
	8	0.12
	9	0.50
Glucose + lysine	3	0.00
	5	0.00
	6	0.16
	7	0.35
	8	0.65
	9	0.84

Absorbances were measured at 500 mμ with a Cary recording spectrophotometer[d] in a 1-cm. cell. Solutions with absorbances greater than 0.8 were diluted before measuring. The results are given in Table 1.

Effect of pH and different concentrations of mixtures of 3 amino acids in glucose solutions. To determine the effect of such multiple factors as pH, mixtures of amino acids, and different concentrations of them on heated dilute solutions of glucose, a statistically designed experiment was conducted. It consisted of a 3x2x4 factorial arrangement with 3 amino acids at 2 concentrations each and 4 different pH's. All solutions contained the same concentration of glucose, 0.025 *M*. The amino acids were DL-alanine, L-glutamic acid monohydrochloride, and L-lysine monohydrochloride. The design was so arranged that each solution contained the 3 acids with the concentration of the individual acids either 0.0025 *M*. or 0.125 *M*., and was buffered at a pH of either 4, 6, 7, or 9.

To reduce the number of samples required to be handled at one time, the design was set up in two parts, each having a factorial of 3x2x2. This was identical with the above 3x2x4 except that pH 4 and 6 were used in one arrangement and pH 7 and 9 in the other. These solutions were made up, heated, and the resultant color measured as in the previous experiment. The absorbances of the solutions relative to distilled water are shown in Table 2.

TABLE 2

Effect of mixtures of amino acids at various levels of concentration on the browning of a 0.025*M*. glucose solution at pH 4, 6, 7 and 9 heated for 20 minutes at 114°C. in an autoclave

Components[1]	Absorbance at 500 mμ			
	pH 4	pH 6	pH 7	pH 9
Glucose	0.00	0.00	0.08	0.26
$A_1Gl_1L_1$	0.00	0.01	0.09	0.29
$A_1Gl_1L_2$	0.00	0.06	0.48	0.93
$A_1Gl_2L_1$	0.00	0.02	0.08	0.23
$A_1Gl_2L_2$	0.00	0.06	0.54	0.97
$A_2Gl_1L_1$	0.00	0.02	0.09	0.23
$A_2Gl_1L_2$	0.00	0.08	0.52	1.20
$A_2Gl_2L_1$	0.00	0.02	0.10	0.28
$A_2Gl_2L_2$	0.00	0.06	0.62	1.1

[1] A_1 = DL-Alanine at 0.0025 *M*. level.
A_2 = DL-Alanine at 0.125 *M*. level.
Gl_1 = L-Glutamic acid monohydrochloride at 0.0025 *M*. level.
Gl_2 = L-Glutamic acid monohydrochloride at 0.125 *M*. level.
L_1 = L-Lysine monohydrochloride at 0.0025 *M*. level.
L_2 = L-Lysine monohydrochloride at 0.125 *M*. level.

RESULTS

Effect of individual amino acids and pH. The amount of color developed in the various glucose solutions is recorded in Table 1 and in Figure 1 as absorbance at 500 mμ. The compounds DL-alanine, an example of the neutral amino acids, and L-glutamic acid monohydrochloride, an example of the acidic amino acids, had no apparent effect on the amount of browning which took place irrespective of the pH of the solution. However, the lysine, an example of the basic amino acids, significantly influenced the amount of color formed. In the solutions containing only glucose no browning occurred below pH 7, but above this point color began to form in increasing amounts as the alkalinity of

[d] Mention of trade names does not imply endorsement by the U. S. Department of Agriculture over similar products not mentioned.

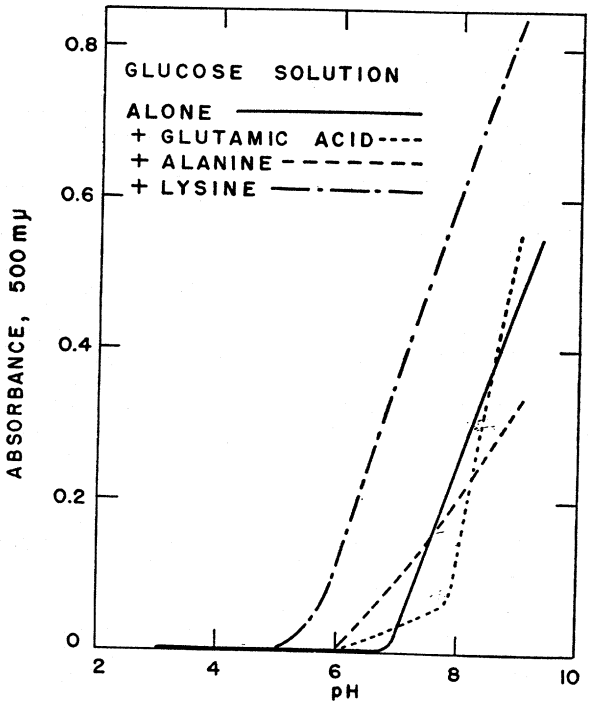

Figure 1. Absorbance at 500 mμ.

the solution increased. When lysine was present with the glucose at pH 7 and above, the solutions were browner than the corresponding controls. Further, this amino acid caused a small amount of color in the glucose solution at pH 6. This is in agreement with the observations made by Joslyn on the browning of orange juice *(3)*. Under the conditions of the present investigation those solutions containing either alanine or glutamic acid were not more colored than the controls; hence it was concluded that the degree of basicity of the solution was a dominant factor in the amount of color produced. This basic condition often occurs in natural products such as maple sirup, but it is a condition which has not been observed in the majority of the studies on browning which have emphasized the effect of pH upon the reaction of amino acids and reducing sugars.

Effect of mixtures of amino acids at different levels of concentration and pH. The data in Table 2 again show the necessity of an alkaline medium for significant color development in dilute solutions. Also, the positive effect of lysine was again demonstrated by the browning which occurred at pH 6, even at a very low level of concentration.

At pH 7 and pH 9 the reaction of the medium plus the effect of the amino acids caused the formation of significant color in all the treatments. Therefore, to determine the relative effects and whether or not there were interactions among the alanine, glutamic acid, lysine and pH, the data obtained by the 16 treatments (Table 2) were analyzed statistically.

The experimental design used was an unreplicated 2^4 factorial as shown in

Table 3. The data were treated by an analysis of variance and the main effects and the first-order interactions were tested using higher-order interactions as experimental error. The complete analysis is shown in Table 4. To attain significance at the 1% probability level, an F value of 16.26 was required. Therefore only the lysine x pH interaction is significant. It is also clear from this table that the effects of lysine and pH are unequivocally the only factors causing the formation of color in the glucose solution. However, because of the lysine x pH interaction these main effects have to be interpreted with caution. It will be helpful, therefore, to look at the table of means for these two factors (Table 5). It is apparent from this table that most of the treatment

TABLE 3

Experimental design of the 2^4 factorial showing combinations of the four factors pH, alanine, glutamic acid, and lysine

	A_1				A_2			
	Gl_1		Gl_2		Gl_1		Gl_2	
	L_1	L_2	L_1	L_2	L_1	L_2	L_1	L_2
pH 7	$7A_1Gl_1L_1$	$7A_1Gl_1L_2$	$7A_1Gl_2L_1$	$7A_1Gl_2L_2$	$7A_2Gl_1L_1$	$7A_2Gl_1L_2$	$7A_2Gl_2L_1$	$7A_2Gl_2L_2$
pH 9	$9A_1Gl_1L_1$	$9A_1Gl_1L_2$	$9A_1Gl_2L_1$	$9A_1Gl_2L_2$	$9A_2Gl_1L_1$	$9A_2Gl_1L_2$	$9A_2Gl_2L_1$	$9A_2Gl_2L_2$

A = Alanine
Gl = Glutamic acid
L = Lysine

Subscript 1 = low level of concentration
Subscript 2 = high level of concentration

TABLE 4

Results of statistical analysis

Source of Variation[1]	Degrees of Freedom	Sums of Squares	Mean Squares	F
Total	15	2.1868	.....	.....
A_1 vs. A_2	1	0.0105	0.0105	3.48
Gl_1 vs. Gl_2	1	0.0008	0.0008	0.27
L_1 vs. L_2	1	1.5563	1.5563	515.33
pH 7 vs. pH 9	1	0.4658	0.4658	154.24
A × Gl	1	0.0008	0.0008	0.27
A × L	1	0.0095	0.0095	3.15
A × pH	1	0.0033	0.0033	1.09
Gl × L	1	0.0011	0.0011	0.10
Gl × pH	1	0.0028	0.0028	0.93
L × pH	1	0.1208	0.1208	40.00
Error	5	0.0151	0.00302	.....
5% level	....	.....	.....	6.61[2]
1% level	....	.....	.....	16.26[2]

[1] Symbols same as in Table 3.
[2] Book value.

TABLE 5

Table of the means of lysine and pH Interaction

	L_1	L_2	Means
pH 7	0.09	0.54	0.32
pH 9	0.26	1.05	0.66
Means	0.18	0.80	

effects can be attributed to the synergistic effect of the lysine-pH complex when the solution is alkaline. There is, of course, definite indication of a pH effect in the absence of lysine and this confirms the data presented in Table 1. Also there was some indication of a lysine effect at pH 7.

All this suggests that a degradation of the sugar may be a primary reaction and a prerequisite in the browning mechanism. In these experiments browning occurred only in alkaline solutions except for those in which lysine was present. In these solutions a small amount of color was formed at a pH as low as 6. This could have been due to a local basicity effect of either one or both of the amino groups. This hypothesis was tested further by determining the effect on browning of two other basic amino acids, arginine and histidine. These, however, had no effect on the amount of browning in a 0.025 *M*. glucose solution. A further study of the increased color production due to the presence of lysine in an alkaline sugar solution will be reported in a subsequent paper.

The glucose solutions that were 0.0025 molar in respect to lysine (Table 2) contained 20 times the amount of nitrogen that is present in maple sap. Further, only a small percentage of the nitrogen in the sap is in the form of amino acids and a still smaller fraction is lysine. Therefore, the fact that no significant increase in color occurred in these solutions with the low-level concentration of amino acids, would be further evidence that amino acids are not responsible for the browning of maple sirup.

SUMMARY

The amount of color developed in a heated dilute glucose solution was influenced by the pH of the solution and type of added amino acid as follows:

1. Glucose solutions containing no amino acids developed color only when their alkalinity exceeded pH 7.
2. An alkaline medium was a prerequisite for significant browning in all cases.
3. The presence of alanine or glutamic acid in the glucose solution did not cause a significant increase in the amount of color produced relative to the control at any pH.
4. The glucose solutions containing lysine exhibited more browning at pH 7 and above than did similar solutions of only glucose. Further, this amino acid induced the formation of a small amount of color at pH 6.
5. The effect of lysine did not appear to be due to its basicity, since arginine and histidine, other basic amino acids, had no positive effect on browning.
6. Statistical analysis of the data shows that lysine and alkaline pH had a synergistic effect on the amount of color developed and that no other combination had such an interaction.

Extrapolation of these results on model systems to the browning of maple sirup indicates that amino acids have no part in this reaction.

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